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Conference Reports

ELECTROPHORESIS SOCIETY MEETING HOSTED AT NBS

by Dennis J. Reeder, Diane K. Hancock, Jesse J. Edwards, and Kristy L. Richie

Center for Analytical Chemistry
National Measurement Laboratory
National Bureau of Standards

The National Bureau of Standards (NBS) hosted the Electrophoresis Society Meeting March 26–28, 1986. The meeting, co-sponsored by the Society and the Center for Analytical Chemistry, drew about 135 scientists. The participants represented industry (40 percent); local, State, and Federal government agencies (40 percent); and universities or university hospitals (20 percent).

The meeting was structured to allow maximum interaction among the participants. Morning plenary sessions were held in the Green Auditorium and afternoon sessions in the Gaithersburg Holiday Inn. Ten companies demonstrated their latest instrumentation in a commercial exhibit.

In the first plenary session, Bruce Budowle of the Forensic Science Research and Training Center, Federal Bureau of Investigation, showed advances in the technology of making ultrathin-layer polyacrylamide gel isoelectric focusing a more reproducible method. The method is becoming a useful tool in forensic analyses and has shown remarkable versatility in its adaptation for genetic analyses of small amounts of human body fluids.

John Fawcett presented a comprehensive and detailed review of comparisons between ordinary isoelectric focusing and electrofocusing in immobilized pH gradients. His work with Andreas Chrambach of the National Institutes of Health (NIH) provided insight into such criteria as resolving power, reproducibility, susceptibility to pH and voltage measurement, ease of protein isolation, preparative load capacity and purity of product.

Carl Merril (NIH) provided a historical background to the process of silver staining of proteins. He gave examples of how detection techniques have progressed from direct observation of protein-coated microspheres and colored proteins, requiring milligram amounts, to the detection of proteins by their ultraviolet absorption, to labelling with radionuclides, to direct staining with organic fluorescent dyes, and most recently to silver stains which require only a tenth of a nanogram. He also demonstrated how most protein stains and autoradiographic methods exhibit protein-specific quantitative optical density/concentration relationships, which are indicative of the dependence of detection methods on the content of specific groups within each protein.

Norman Anderson of Proteus Technologies, Inc., spoke on the subject of future directions of electrophoresis, with particular emphasis on the role that electrophoresis will play in biotechnology application areas that include exploring the human genome and newer areas relating to improvements in plant breeding and production of better plant products.

Philip Serwer from the University of Texas Health Science Center, San Antonio, detailed the separation of viruses and viral components in extracts of infected cells by use of agarose gel electrophoresis. This technique is comparatively inexpensive and nonselective for the detection of particles in either unfractionated or partially-

fractionated lysates of infected cells. Spheres can be discriminated from rods by measurement of electrophoretic mobility as a function of gel concentration. Dr. Serwer also showed the use of agarose in two-dimensional applications.

Nancy Stellwagen, University of Iowa, concluded the plenary sessions by summarizing the current interest in gel electrophoresis of DNA molecules. Of particular interest was her description of the use of pulsed electric fields for the separation of chromosome-sized molecules of DNA. Using alternating inhomogeneous electric fields for separation and the application of electric birefringence to study the orientation of DNA molecules, Dr. Stellwagen researched several theories on the process of DNA separation. In particular, the reptation theory for electrophoresis based in the axial movement ("snaking") of DNA molecules through gels seems to have validity and can be expressed in appropriate mathematical equations.

. . . pulsed electric fields for the separation of chromosome-sized molecules of DNA . . .

Although not a formal presentation, the review paper by Susan Olson and Carl Merril was included in the Proceedings publication because of its timely and detailed information regarding the separation of peptides on different media. The extensive bibliography should be useful to anyone beginning work with this technology.

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The afternoon meetings were held in an informal setting in order to give the participants ample opportunity to interact and discuss their findings. The clinical session was combined with the genetics session to yield a lively and interesting session dealing with diagnostic capabilities of electrophoresis. Of note was the series of talks given by the University of Michigan researchers led by Samir Hanash. Dr. Hanash made extensive use of two-dimensional polyacrylamide gel electrophoresis in the study of acute lymphoblastic leukemia. One particular polypeptide designated L3 with a molecular weight of 29,000 showed substantial variability in the staining intensity depending upon the patient being tested. If the spot was intense, very few of the patients relapsed, if the spot was weak, there was a strong likelihood of an unfavorable outcome in the disease process. Thus, detailed analysis of the polypeptide constituents of leukemic cells, made possible by two-dimensional electrophoresis, has the potential of aiding physicians in the treatment of lymphoblastic leukemia.

In the gel media session, participants presented results of their work with newer types of gel media. In particular, a recently introduced derivatized agarose called "Nufix Glyoxyl Agarose" (FMC-Marine Colloids) was discussed. The wide spectrum of glyoxyl agarose properties were presented, including its controllable reactivity toward primary and secondary amines, its native fixation capabilities, and its ability to be cast in any form. A glyoxyl agarose/polyacrylamide gel composite permits in situ immobilization of polypeptides after electrophoretic separation. The application of glyoxyl agarose to blotting and chromatography was also discussed. Also reported was the effect of borate buffers on increased sieving properties of agarose gels. This sieving increase is most pronounced if the borate is present during casting of the gel. Borate also alters agarose viscosity and gel strength, as well as gelling and melting temperatures. Recently reported research on the properties of rehydratable gels has now come to a point of potential standardization. A report was presented by Kristy Richie of NBS on a interlaboratory comparison study of rehydratable gel reproducibility with standard protein preparations. In comparing laboratory results, she noted that there were distinguishable variations in the protein band patterns of the same protein due to several factors in the electrophoretic run. Factors such as electrode separation distance, composition of catholyte and anolyte, temperature, humidity, and time of the electrophoretic run, all influenced the electrophoretic patterns. The second round robin analysis will include more instructions and details for controlling the influencing factors.

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Philippe Arnaud (NIH) compared commonly used nitrocellulose membrane transfer material with the newer zeta probe membranes made of Nylon. His data demonstrated the possibility of "leakage" of a protein of interest from nitrocellulose, but transferred with much tighter binding to the zeta probe membrane. In particular, although different

higher weight molecular forms of alpha$_1$-acid glycoprotein (A_1S) transfer and bind with nitrocellulose, the monomer did not appear to transfer. However, when the zeta probe material was used, the monomer was clearly transferred from the polyacrylamide gel slab and represented the major molecular species of A_1S.

The round table discussion on immobilized pH gradients (IPG) centered on sharing ingenious methods for circumventing various problems encountered with the technique. One such problem is that of solubilizing proteins so that they could enter into the gel matrix. Some laboratories found that addition of 2 mol/L urea to the sample or to the equilibration solution was a useful procedure, while other researchers added carboydrate such as dextrans or agarose to the gel. Some proteins can be applied to the gel surface while others need to be applied to the holes cut into the gel. Examples of analytical and preparative one-dimensional applications were presented as well as two-dimensional applications. Angelika Görg (Technische Universität München) showed excellent resolution of human plasma proteins in a two-dimensional system that utilized immobilized pH gradients as the first dimension. There is continuing evidence that many of the early problems and pitfalls encountered when using immobilized pH gradient technology are now being solved. Examples of very high resolution capabilities were demonstrated.

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In the session on two-dimensional electrophoresis, the leading topic discussion centered around the requirements for data reduction of protein patterns generated by two-dimensional electrophoresis. Guidelines presented for the development of a model protein database were followed by an assessment of the functions and effective uses of such a system as a medium for communicating information. These presentations stimulated much discussion about the types of experiments that are necessary to produce the maximum amount of useful data. Problems associated with data reduction of protein patterns became apparent when approaches to the methods of spot detection and quantification (e.g., parametric versus non-parametric analysis) were presented. This led to extensive discussion of the theoretical and practical aspects of image analysis. Problems of standardization in both isoelectric focusing and SDS-electrophoresis were addressed with emphasis upon the need for commercially-available products of high purity and good reproducibility. The reliability of marker proteins, the acceptable limitations for purity and reproducibility and the problems of production and storage were discussed. It was encouraging to hear of the high priority that commercial firms are placing on standardization of products and techniques. The results of these efforts were shown in the presentation of cleaner molecular weight markers and high quality carbamylated charge trains for isoelectric focusing. Finally, procedures for sample preparation and evaluation were discussed. Data were presented which evaluated sample preparation by ultrafiltration and diafiltration using centrifugal microconcentrators. Also, a novel total protein assay using UV-difference spectroscopy was presented. Results compared favorably with other protein assay methods such as Coomassie dye binding, bicinchoninic acid and the so-called "Lowry" method.

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Current interest in protein detection techniques were aptly displayed by the attendees of the staining session. Joseph Yudelson (Kodak), developer of the nickel protein stain, presented information on the chemistry, nucleation, and amplification process in the nickel and silver visualization systems. These systems which are akin to physical development in the photographic field require formation of critical nucleation clusters if grain formation is to occur. In the case of the nickel stain, palladium serves as the nucleation agent, causing the protein to become a catalytic development center. In the case of silver stain, a much larger number of species (metals, metal oxides, and sulfides) serve as nucleation agents. The stronger the reducing agent in the developer, the smaller the critical cluster size required for the metal reduction to proceed autocatalytically. Hence the alkaline formaldehyde reducer used in the silver stain requires only approximately two silver atoms for critical cluster size while the dimethylamine borane used in the nickel stain requires a larger critical cluster size. These differences account for the greater instability of the silver physical developer but also for its somewhat greater sensitivity. Dr.

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Yudelson presented calculations indicating that the silver stain is presently near its limit of sensitivity.

Diane Hancock (NBS) spoke on efforts to probe the mechanism of the silver stain with the goal of establishing staining conditions to increase sensitivity, and to decrease background staining. Data from various preliminary silver-protein binding studies were presented and discussed. Binding ratios on the order of 25 to 1 were found for the titrations of bovine serum albumin with $AgNO_3$ (silver nitrate) where silver concentrations were monitored with a silver ion specific electrode. Neutron activation analysis was used to determine silver distribution in polyacrylamide slab gels stained with either a Merril-type silver stain or with an ammonical silver stain. Highest silver concentrations were found in the stained protein bands, though substantial silver was present throughout the gel and the lowest concentrations were found in areas of negative staining. Results of feasibility studies using ^{109}Ag Nuclear Magnetic Resonance spectrometry (NMR) to identify silver-protein binding sites were presented. The data indicated that solution dynamics were such that little useful information could be obtained with this technique.

Betty Mansfield (Oak Ridge National Laboratory) presented a fluorescence assay method for determining 2,5-diphenyloxazole (PPO) concentrations from scintillation fluids. This analysis results in a considerable savings of time as the solution can now be regenerated many times before the expensive PPO must be precipitated, filtered, and dried.

The final sessions devoted to nucleic acids and particle electrophoresis brought together experts who focused their attention on physical characterization of materials separated in agarose gel electrophoresis. Considerable attentioan was given to a system developed by Douglas Gersten, Georgetown University, for automated nucleic acid electrophoresis and hybridization analysis.

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The Electrophoresis Society Workshop was successful in bringing together authorities who could demonstrate the latest techniques in electrophoresis. Participants observed ultrathin methods for forensic analyses presented by Bruce Budowle and Dale Dykes. Robert Allen and Calvin Saravis showed how electrophoresis and enzyme staining could be performed very rapidly on the same apparatus. Comparisons of equipment capabilities using the same samples were demonstrated by Hoefer Scientific Instruments and Haake Buchler Instruments. The new "Phast" system for high-performance electrophoresis was demonstrated by Pharmacia, Inc.

Commercial workshops held during the first two days of the meeting were also well attended. Hoefer Scientific Instruments presented a workshop on minigel electrophoresis and Western blotting. Protein Databases demonstrated the use of a new system, "PDQuest" for 2-D Gel Analysis. Pharmacia, Inc., introduced the "Phast System" for high-performance electrophoresis to the participants. This highly automated system had only been introduced to the scientific community at a meeting in Washington, DC, two days before the Society meeting. LKB, Inc., presented topics on electrofocusing and immobilized pH gradients. Angelika Görg, a renowned expert in the field, provided interesting insights into the separations process.

In summary, the meeting provided excellent opportunities to learn of the newest techniques and equipment in electrophoresis. It probed the diversity of directions that the field of electrophoresis is taking, hosting numerous stimulating discussions and problem solving sessions along the way. As a beneficial result of the meeting, approaches to problems developed as industrial manufacturers were exposed to the challenges encountered by the researchers using electrophoresis.

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